Journal of Infection and Public Health (2014) 7, 496—507

http://www.elsevier.com/locate/jiph

Prevalence of two multidrug-resistant *Klebsiella* species in an Indian teaching hospital and adjoining community

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Received 9 February 2014; received in revised form 24 March 2014; accepted 24 May 2014

KEYWORDS
Klebsiella species;
ESBL;
Fluoroquinolone resistance;
Multidrug resistance;
Hospital and community acquired

Summary
Background: The Gram-negative pathogenic bacteria *Klebsiella oxytoca* and *Klebsiella pneumoniae* produce the extended spectrum β-lactamase (ESBL) and cephalosporinase enzymes and are the major causes of hospital acquired (HA) infections and epidemics in non-hygienic communities in the majority of developing countries.
Methods: The prevalence of multidrug resistance among 445 strains of *K. oxytoca* and *K. pneumoniae* isolated from clinical samples of patients with gastrointestinal infections over a period of 42 months in the hospital was recorded, along with the sensitivity patterns to 23 antibiotics, including third-generation cephalosporin and fluoroquinolone antibiotics, using the disk-diffusion method.
Results: Of 175 *K. oxytoca* isolates, 143 were ESBL positive and 117 were fluoroquinolone resistant. Of 270 *K. pneumoniae* isolates, 200 were ESBL positive and 195 were independently fluoroquinolone resistant. The HA samples yielded more isolates than the community acquired (CA) samples for each species. The *K. oxytoca* strains were resistant to cefepime, gatifloxacin, ciprofloxacin, ceftazidime, levofloxacin and imipenem, whereas the *K. pneumoniae* strains were highly resistant to ampicillin, norfloxacin, ciprofloxacin, gatifloxacin, ofloxacin, amoxyclav, ceftazidime, cefepime, cefixime, piperacillin and imipenem. The ESBL-producing and fluoroquinolone-resistant *K. pneumoniae* strains were more prevalent than the *K. oxytoca* strains in the HA/CA samples. The minimum inhibitory concentration values of the third-generation cephalosporins: cefotaxime and ceftazidime and the

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http://dx.doi.org/10.1016/j.jiph.2014.05.002

fluoroquinolones: ciprofloxacin and levofloxacin against both species of *Klebsiella* confirmed the resistance in the current/coveted treatment options.
Conclusions: Patients with other bacterial infections had a relatively higher probability of infection with ESBL-producing and fluoroquinolone-resistant *Klebsiella* strains. The data presented here highlight the alarming state of *Klebsiella* infection dynamics in the hospital and adjoining communities.

Introduction

Surveillance of the Gram-negative (GN) pathogenic bacteria *Klebsiella oxytoca* and *Klebsiella pneumoniae* (tribe Klebsiellae, family Enterobacteriaceae) that produce extended spectrum β-lactamase (ESBL) and cephalosporinase enzymes is a responsibility of hospital management. These enzymes produced by several strains of both species cause resistance to penicillin derivatives including the third-generation cephalosporins (3GCs) and monobactams, rendering these species notorious pathogens [1—3]. Control measures remain difficult for a clinician/hospital epidemiologist depending on the extent of drug resistance to other classes of antibiotics, both empirically and routinely after ascertaining the antibiograms of isolates *in vitro*. By producing ESBL and cephalosporinase enzymes and also using other mechanisms, multidrug-resistant (MDR) *Klebsiella* strains cause frequent grievous bacteremia/septicemia. For example, fatal neonatal septicemia caused by a metallo-β-lactamase-producing *K. pneumoniae* strain was reported locally [4]. Indeed, GN bacteria resistant to all major classes of antibiotics have emerged as intense pandrug-resistant (PDR) bacteria. PDR strains of *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *K. pneumoniae* cause comorbidities and immature mortality [5]. However, the term PDR has not yet reached a consensus in the literature, despite its clear etymological meanings, 'resistant to almost all commercially available antimicrobials', 'resistant to all antimicrobials routinely tested' and 'resistant to all antibiotic classes available for empiric treatment'. *Klebsiella* sp. colonize the respiratory, gastrointestinal and urinary tracts.

The variant CTX-M (resistant to cefotaxime) type β-lactamase is the predominant ESBL variant, compared to the other variants, such as temoneira (TEM) and sulfhydryl variable (SHV) types, of ESBLs [6]. These two *Klebsiella* species, along with *Escherichia coli* and *Proteus mirabilis* and a few members of the Enterobacteriaceae, such as *Citrobacter* sp., *Serratia* sp. and *Morganella morganii* with ESBL-producing capacity, have been widely reported and cause longer hospitalization, comorbidities and fatality with frequent failures of empiric therapy [3,7—9]. Therefore, ESBL production is now widespread in the family.

For the control of Enterobacteriaceae pathogens, a cephalosporin antibiotic, such as cefepime, ceftriaxone, cefotaxime, ceftazidime, cefpodoxime and cefixime, or fluoroquinolones, such as gatifloxacin, moxifloxacin, ciprofloxacin, levofloxacin, temafloxacin, grepafloxacin and trovafloxacin, are often used empirically. Fluoroquinolones are effective for bacterial control by interfering with the bacterial DNA gyrase and topoisomerase IV. These broad-spectrum antibiotics are even used against *Staphylococcus aureus* and other Gram-positive (GP) bacteria. Because of the adverse effects on the host caused by the latter three fluoroquinolones, new antibiotics are continually developed. However, resistance to fluoroquinolones by Enterobacteriaceae members is widespread [10]. The resistance mechanisms include alterations in the drug target and/or in the permeation of the drug to reach the target. Additionally, enrofloxacin (fluoroquinolone) is degraded by the fungus *Gloeophyllum striatum* in mixed infections [11]. Enrofloxacin metabolism by the fungus *Mucor ramannianus* was also reported. During a 21-day incubation, 78% of the enrofloxacin was transformed into three metabolites, namely enrofloxacin *N*-oxide (62%), *N*-acetyl ciprofloxacin (8.0%), and desethylene-enrofloxacin (3.5%). The major metabolite of enrofloxacin in animals is ciprofloxacin, produced by N-deethylation of the ethylpiperazine ring [12]. For example, the majority of strains of 11 different GN bacteria isolated from rectal swabs from a large number of patients in the intensive care unit (ICU) of a hospital in Istanbul revealed that 40% of the isolates were resistant to ciprofloxacin. The study determined that plasmid-mediated quinolone-resistant determinants, namely *qnrB1*, *qnrS1*, were isolated from the isolates [13].

Moreover, approximately 150 types of ESBL bacterial strains have been described with a worldwide distribution, clearly demonstrating that β-lactam

antibiotic resistance emerged in geographic zones where the particular antibiotic was first used [14,15]. Specifically, in a study with *K. pneumoniae*, it was reported that ciprofloxacin use and ESBL production are related [15], confirming that the use of a particular antibiotic induces resistance to it in the target pathogen. Furthermore, Enterobacteriaceae that cause urinary tract infections (UTIs) that are resistant to fluoroquinolone and cephem are often obtained from the urine samples of women with cases of uncomplicated UTI [16]. Therefore, the extent of multidrug resistance in any pathogen is a significant obstacle for control *in vivo*. Unfortunately, virulent enteric bacteria such as *Klebsiella* sp. and *E. coli* are present in non-hygienic dwellings, and infection with these bacteria is often the cause of high infant mortality and outbreaks of infrequent epidemics in developing countries [17].

The current study provides systematic information on 445 strains of two species of *Klebsiella* isolated from clinical samples of patients with gastrointestinal infections over a period of 42 months in a hospital. The antibiotic-resistant patterns for the 23 antibiotics used in the study indicate the alarming state of the infection dynamics of MDR strains of *K. oxytoca* and *K. pneumoniae* in a central Indian state. In particular, resistance to cephalosporin and fluoroquinolone antibiotics is reported in this study. Clearly, surveillance in a hospital and identification of the organism causing an ailment is necessary for clinical stewardship. This Indian state is more poverty-stricken than the national average because of urban-slum ghettos and under-developed aboriginal pockets. This study identifies the actual prevalence of enteropathogens that cause significant under-5 mortality and life-threatening situations in adults [14]. Furthermore, this study is as an example of research that cannot usually be performed unless one is a clinical microbiologist. This work should help revise the antibiotic policy of the state.

Methods

Isolation, identification and antibiotic sensitivity of bacterial isolates

Over 42 months (July 2010—December 2013), a total of 1645 clinical samples, including urine, stool and rectal swabs, were collected from hospitalized patients as hospital acquired (HA, patients who were admitted for more than 48 h with no prior enteropathogenic infection) samples and outdoor patients as community acquired (CA). The CA patients complained of diarrhea, dysentery and other associated symptoms. The samples were sent directly to the department of microbiology within 30 min of collection for culturing, identification and monitoring of antibiotic sensitivity. The isolated strains were identified by standard biochemical tests. Antibiotic susceptibility tests were performed using the Kirby—Bauer disk diffusion method [14]. Strain no. 2275 of *K. oxytoca* and strain no. 4031 of *K. pneumoniae* from the Microbial Type Culture Collection (MTCC) were used as reference strains.

Detection of ESBL producers

A double-disk-diffusion-synergy test was used for the determination of ESBL producers in both *Klebsiella* species. In this test, synergy was determined between a disk of augmentin (a combination of 20 g amoxicillin and 10 g clavulanic acid) and two discs of the third-generation cephalosporin antibiotics ceftazidime (30 g) and cefotaxime (30 g), all placed an equidistant 30 mm apart on the lawn culture of a test bacterium on a Mueller-Hinton (MH, HiMedia) agar plate in duplicate. The test bacterium was considered ESBL producing when the inhibition-zone size around both/one test antibiotic discs increased toward the augmentin disk because the clavulanic acid in the augmentin disk was inactivated by the ESBL enzyme produced by the test bacterium [18].

Determination of MIC values

For confirmation and quantitative analysis of the ESBL strains, a 96-well micro-titer plate was used to determine the minimum inhibitory concentration (MIC) values of the third-generation cephalosporins cefotaxime and ceftazidime in broth cultures for 100 selected MDR strains of *K. oxytoca* and *K. pneumoniae* each, of which 50 were ESBL producers and 50 were non-ESBL producers. An exponential culture of a test strain in MH broth was diluted with normal saline to obtain a level equivalent to the 0.5 McFarland standards. To an aliquot of 20 μl of overnight grown test culture, an aliquot of 100 μl antibiotic (cefotaxime or ceftazidime) stock solution of 1024 μg/ml and 0.1 ml MH broth were added to the second well of the micro-titer plate. These solutions were two-fold serially diluted in each adjacent well until a final concentration of 0.25 μg/ml antibiotic was achieved in the 12th well. Finally, 5 μl of 0.5% 2,3,5-triphenyltetrazolium chloride (TTC) was added as an indicator of bacterial growth, and the micro-titer plate was incubated at 37 °C for 18 h. The

Table 1 Occurrence of *Klebsiella oxytoca* and *Klebsiella pneumoniae* in clinical samples obtained during January 2010—June 2013.

Period	*K. oxytoca*		*K. pneumoniae*	
	HA	CA	HA	CA
Jul 2010—Dec 2010	19 (15) [13]	15 (09) [11]	22 (15) [19]	17 (15) [10]
Jan 2011—Jun 2011	11 (05) [06]	09 (04) [04]	28 (19) [23]	19 (11) [08]
Jul 2011—Dec 2011	15 (07) [11]	12 (09) [07]	30 (22) [18]	17 (12) [11]
Jan 2012—Jun 2012	12 (07) [08]	03 (03) [02]	27 (22) [20]	10 (10) [03]
Jul 2012—Dec 2012	16 (11) [12]	12 (05) [07]	18 (11) [13]	18 (09) [14]
Jan 2013—Jun 2013	18 (12) [14]	11 (04) [08]	21 (14) [14]	13 (17) [18]
Jul 2013—Dec 2013	15 (10) [07]	07 (03) [07]	15 (10) [13]	15 (13) [11]
Total	106 (67) [71]	69 (37) [46]	161 (113) [120]	109 (87) [75]

HA, hospital acquired; CA, community acquired. Numbers in parenthesis represent number of ESBL producing isolates; numbers in square bracket represent number of fluoroquinolone resistant isolates.

wells were examined for the absence of pink color (bacterial growth) in a well with the specific dilution, which was recorded as the MIC value. The first well of the micro-titer plate was the control without any antibiotic solution. The results were interpreted basing on the revised break-point values of the third-generation cephalosporin antibiotics according to specifications of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2013 [19]. The reference strain and all isolated *K. pneumoniae* and *K. oxytoca* strains were subjected to antibiotic sensitivity tests using Kirby—Bauer's or the disk diffusion method with MH agar as detailed previously [20]. Similarly, the MIC values of the two frequently used fluoroquinolone antibiotics ciprofloxacin and levofloxacin against 50 drug-sensitive strains and 50 resistant strains of *K. oxytoca* and *K. pneumoniae* were also determined.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Medical Science version 17.0 (SPSS Inc., IL).

Results

Isolation of *K. oxytoca* and *K. pneumoniae* strains

A total of 175 strains of *K. oxytoca* were isolated from clinical samples, of which 106 isolates were from hospitalized patients and 69 isolates were CA samples. Of the 106 HA strains, 67 isolates were ESBL positive and 71 strains were independently resistant to the fluoroquinolone antibiotics. Similarly, of the 69 CA *K. oxytoca* strains, 37 isolates were ESBL-producing strains and 46 strains were independently fluoroquinolone resistant (Table 1). A total of 270 strains of *K. pneumoniae* were isolated, of which 161 isolates were from HA samples and 109 isolates were from CA samples. Several of the 270 isolates were also resistant to *K. oxytoca*. Of the 161 HA strains, 113 *K. pneumoniae* isolates were ESBL-producing strains and 120 strains were independently fluoroquinolone antibiotic resistant. Similarly, of the 109 CA *K. pneumoniae* strains, 87 isolates were ESBL-producing strains and 75 strains were independently resistant to the fluoroquinolone antibiotics (Table 1).

Biochemical identification of *K. oxytoca* and *K. pneumoniae* strains

K. oxytoca was identified based on the colony characteristics on cysteine lactose electrolyte-deficient (CLED) and MacConkey agar, together with the results of 9 biochemical tests. Yellow mucoid colonies on CLED agar and pink-colored mucoid colonies (Fig. 1) on MacConkey agar were noted because of lactose fermentation. Furthermore, positive results for catalase, indole, Voges—Proskauer, citrate urease and nitrate reduction tests and negative results for oxidase and methyl-red were confirmatory. For the triple sugar iron (TSI) test, the bacterium only produced acid, but no gas (Table 2). Similarly, the *K. pneumoniae* (Fig. 2) strains were identified based on the colony and biochemical characteristics as described (Table 2). Both strains were non-motile.

Antibiotic susceptibility patterns of *K. oxytoca* and *K. pneumoniae* strains

The antibiotic susceptibility patterns of the isolated *K. oxytoca* and *K. pneumoniae* strains were studied over 42 months using 23 antibiotics. The

Table 2 Microbiological identification of the isolated clinical strains of *K. oxytoca* and *K. pneumoniae*.

Isolated bacteria	MTCC strain	Media	Colony characters	Biochemical characters
K. oxytoca	2275	CLED agar	Yellow, mucoid	Positive for catalase, indole, VP, citrate, urease and nitrate tests. Negative for oxidase and MR tests.
		MacConkey agar	LF, pink, mucoid	TSI: A/AG; motility was negative
K. pneumoniae	4031	CLED agar	Yellow, mucoid	Positive for catalase, VP, citrate, urease and nitrate tests. Negative for oxidase, indole and MR tests. TSI: A/AG; motility was negative
		MC agar	LF, pink, mucoid	

MR, methyl red; CLED, cysteine lactose electrolyte deficient; LF, lactose fermenting; MTCC, microbial type culture collection; VP, Voges—Proskauer; TSI, triple sugar iron; A/A, acid production both in slant and butt; A/AG, acid production both in slant and butt with gas.

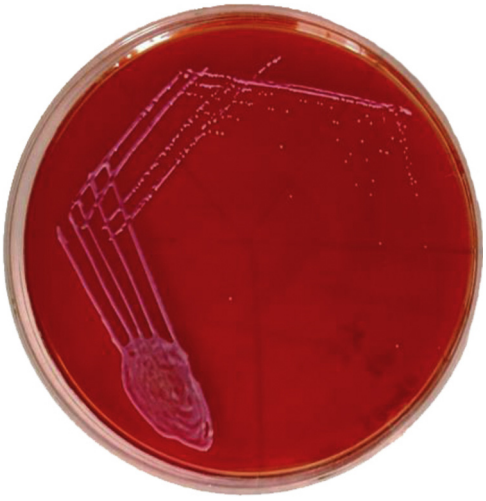

Figure 1 Pink, mucoid, lactose fermenting colonies of *K. oxytoca* on MacConkey agar.

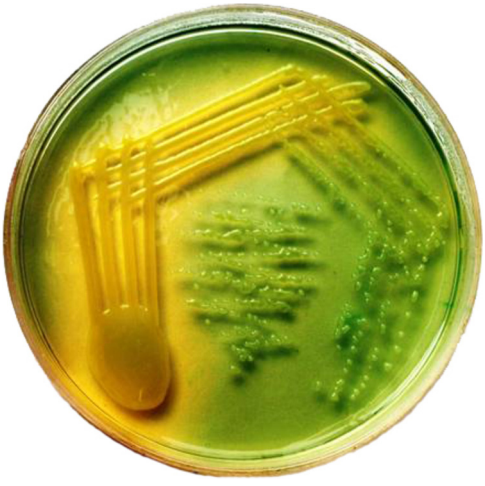

Figure 2 Yellow, mucoid, lactose fermenting colonies of *K. pneumoniae* on CLED agar.

HA *K. oxytoca* strains were highly resistant to cefepime (89%), followed by gatifloxacin (82%), ciprofloxacin (78%), and ceftazidime (76%) and were least resistant to imipenem (23%). Similarly, the CA *K. oxytoca* isolates were highly resistant to ciprofloxacin (79%), followed by gatifloxacin (77%), cefixime (74%), and cefepime (72%) and were least resistant to imipenem (15%) and levofloxacin (21%) (Table 3). The HA *K. pneumoniae* strains were highly resistant to ampicillin (85%), followed by norfloxacin (84%), gatifloxacin (83%), ofloxacin (83%), amoxyclav (82%), and cefepime (81%) and were least resistant to imipenem (21%). The CA *K. pneumoniae* strains were also highly resistant to ceftazidime (79%), followed by the three fluoroquinolone antibiotics, ciprofloxacin, gatifloxacin, and norfloxacin (all at 74%), cefepime, cefixime, and piperacillin (all at 71%) and were the least resistant to imipenem (19%). The resistance percentage values clearly indicate the occurrence of ESBL and fluoroquinolone-resistant strains for both *K. oxytoca* and *K. pneumoniae* isolates in the hospital and community settings. Moreover, the resistance to imipenem and meropenem (carbapenem antibiotics) further complicates the treatment strategy of a clinician because these are the most recent generation of broad-spectrum antibiotics used against drug-resistant pathogenic bacteria and for the empiric therapy of critically ill patients.

MIC values

With a cohort of 50 ESBL-producing *K. oxytoca* strains, the MIC range for cefotaxime was 64—256 μg/ml for 43 strains, and the remaining 7 strains had MIC values ≥512 μg/ml. Similarly, for ceftazidime, the MIC range was 32—256 μg/ml for 32 strains, and the remaining 18 strains had MIC values ≥512 μg/ml. These MIC values confirmed

Table 3 Percentage of resistance of *K. oxytoca* and *K. pneumoniae* to 23 antibiotics of different classes.

Antibiotic group	Antibiotics (μg/disk)	Resistance (in %)			
		K. oxytoca		*K. pneumoniae*	
		HA	CA	HA	CA
Aminoglycosides	Amikacin 30	51	39	74	57
	Gentamicin 10	48	36	79	48
	Netillin 30	52	48	46	35
β-Lactams	Amoxyclav 30	62	38	82	70
	Ampicillin 10	67	43	85	71
	Piperacillin 100	71	62	76	67
	Piperacillin/tazobactam 100/10	49	35	67	49
Carbapenems	Imipenem 10	23	15	21	19
	Meropenem 10	45	49	36	31
Cephalosporins	Cefepime 30	89	72	81	71
	Cefixime 30	68	74	66	71
	Cefotaxime 30	64	47	62	57
	Ceftazidime 30	76	58	65	79
	Ceftriaxone 30	42	37	56	33
	Cefuroxime 30	58	45	69	54
Fluoroquinolones	Ciprofloxacin 5	78	79	80	74
	Gatifloxacin 5	82	77	83	74
	Levofloxacin 5	30	21	28	22
	Norfloxacin 10	63	35	84	74
	Ofloxacin 5	71	63	83	66
Monobactam	Aztreonam 30	46	33	76	43
Sulfonamides	Co-trimoxazole 25	51	45	59	45
Synthetic drug	Nitrofurantoin 300	48	35	71	49

HA, hospital acquired; CA, community acquired.

the presence of ESBL-producing strains because the breakpoint for cefotaxime and ceftazidime is ≥2 and ≥4 μg/ml, respectively [19]. Similarly, all 50 non-ESBL *K. oxytoca* isolates were in the MIC range of 0.5 to 4.0 μg/ml for both cefotaxime and ceftazidime (Table 4). For the 50 ESBL-producing *K. pneumoniae* strains, the MIC range for cefotaxime was 64 to 256 μg/ml for 24 strains, and the

Table 4 Detection of MIC values in 50 ESBL producing *K. oxytoca* and *K. pneumoniae* strains with 2 third-generation cephalosporins used in DDST.

Breakpoint (μg/ml)	*K. oxytoca*				*K. pneumoniae*			
	Cefotaxime		Ceftazidime		Cefotaxime		Ceftazidime	
	ESBL positive (*n* = 50)	ESBL negative (*n* = 50)	ESBL positive (*n* = 50)	ESBL negative (*n* = 50)	ESBL positive (*n* = 50)	ESBL negative (*n* = 50)	ESBL positive (*n* = 50)	ESBL negative (*n* = 50)
≤0.25	—	01	—	—	—	—	—	—
0.5	—	04	—	15	—	11	—	17
1	—	10	—	18	—	21	—	19
2	—	19	—	06	—	18	—	12
4	—	16	—	06	—	—	—	—
8	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—
32	—	—	14	—	—	—	—	—
64	12	—	06	—	06	—	23	—
128	18	—	02	—	08	—	07	—
256	13	—	10	—	10	—	12	—
≥512	07	—	18	—	26	—	08	—

DDST, double disk synergy test.

Table 5 Detection of MIC values in 50 fluoroquinolone resistant producing *K. oxytoca* and *K. pneumoniae* strains with two frequently used fluoroquinolone antibiotics.

Breakpoint (μg/ml)	*K. oxytoca*				*K. pneumoniae*			
	Ciprofloxacin		Levofloxacin		Ciprofloxacin		Levofloxacin	
	Sensitive ($n=50$)	Resistant ($n=50$)	Sensitive ($n=50$)	Resistant ($n=50$)	Sensitive ($n=50$)	Resistant ($n=50$)	Sensitive ($n=50$)	Resistant ($n=50$)
≤0.25	25	—	09	—	18	—	21	—
0.5	17	—	13	—	11	—	17	—
1	08	—	28	—	21	—	12	—
2	—	03	—	—	—	—	—	—
4	—	09	—	20	—	—	—	—
8	—	05	—	03	—	15	—	06
16	—	10	—	—	—	10	—	—
32	—	—	—	—	—	03	—	—
64	—	16	—	09	—	09	—	29
128	—	02	—	13	—	01	—	12
256	—	01	—	—	—	10	—	03
≥512	—	04	—	—	—	02	—	—

remaining 26 strains had MIC values ≥512 μg/ml. Similarly, for ceftazidime, the MIC range was 42 to 256 μg/ml for 32 strains, and the remaining 8 strains had MIC values ≥512 μg/ml. All 50 non-ESBL *K. pneumoniae* strains were in the MIC range of 0.5 to 2.0 μg/ml for both cefotaxime and ceftazidime. Therefore, all 50 strains were confirmed to be susceptible to both antibiotics (Table 4).

Furthermore, with the 50 fluoroquinolone sensitive *K. oxytoca* strains, the MIC range for ciprofloxacin was 0.5—1.0 μg/ml for 25 strains, and the remaining 25 strains had MIC values ≤0.25 μg/ml. For levofloxacin, the MIC range was 0.5—1.0 μg/ml for 28 strains, and the remaining 22 strains had MIC values of ≤0.25 μg/ml. These MIC values confirmed the susceptibility of these 50 strains to the fluoroquinolone antibiotics because the breakpoints for ciprofloxacin and levofloxacin are ≥1 and ≥2 μg/ml, respectively. Of the 50 fluoroquinolone-resistant *K. oxytoca* strains, 46 isolates had a MIC value ranging from 2 to 256 μg/ml, and the remaining 4 isolates had a MIC value ≥512 μg/ml for ciprofloxacin (Table 5). For the 50 fluoroquinolone sensitive *K. pneumoniae* strains, the MIC range for ciprofloxacin was 0.5—1.0 μg/ml for 32 strains, and the remaining 18 strains had MIC values ≤0.25 μg/ml. For levofloxacin, the MIC range was 0.5—1.0 μg/ml for 29 strains, and the remaining 21 strains had MIC values of ≤0.25 μg/ml. For ciprofloxacin, of the 50 fluoroquinolone-resistant *K. pneumoniae* strains, 48 isolates had a MIC value ranging from 2 to 256 μg/ml, and the remaining 2 isolates had a MIC value ≥512 μg/ml. For levofloxacin, all 50 fluoroquinolone-resistant *K. pneumoniae* strains had MIC values in the range of 8—256 μg/ml (Table 5).

Univariate analysis

The univariate analysis of the *K. oxytoca* ESBL positive and negative isolates showed that the acquisition of ESBL positive strains in a hospital setting was 1.4858 times more likely than from the community (Table 6). This value was 0.5953 times more likely for hospital settings for *K. pneumoniae* (Table 7). Furthermore, males had 1.1693 times more chance of becoming infected with *K. oxytoca* than females (Table 6). Males were also 0.8963 times more likely than females to become infected with *K. pneumoniae* (Table 7). Both HA/CA and male/female pairs of data were statistically insignificant. Additionally, comorbidities from non-infectious ailments were 1.8261 and 4.0594 times more likely for ESBL infections with any *Klebsiella* species. Patients with infections were 0.1934 times more likely to become infected with an ESBL strain for *K. oxytoca*, whereas they were 0.0466 times more likely to become infected with *K. pneumoniae* (Table 7). These values were statistically insignificant for *K. oxytoca* with non-infectious comorbidities, whereas they were significant for *K. pneumoniae*. Similarly, patients with infectious ailments were likely to be infected with ESBL positive bacteria at highly significant levels (Tables 6 and 7).

For the fluoroquinolone-resistant and -sensitive strains, *K. oxytoca* was 1.0143 times more likely to cause infection in a patient in the hospital setting, and a similar likelihood of 1.3268 was determined

Table 6 Univariate analysis of ESBL positive and negative isolates of *K. oxytoca*.

Variables		ESBL positive	ESBL negative	*p*-value	Odd ratio	Range
Strains	HA	67	39	0.2079*	1.4858	0.8023
	CA	37	32			−2.7515
Sex	Male	71	46	0.6311*	1.1693	0.6175
	Female	33	25			−2.2143
Comorbidities	Present	58	29	0.0535*	1.8261	0.9909
	Absent	46	42			−3.3651
Other infections	Present	36	52	<0.0001	0.1934	0.0997
	Absent	68	19			−0.3753

* Statistically not significant.

Table 7 Univariate analysis of ESBL positive and negative isolates of *K. pneumoniae*.

Variables		ESBL positive	ESBL negative	*p*-value	Odd ratio	Range
Strains	HA	113	48	0.0780*	0.5953	0.3343
	CA	87	22			−1.0599
Sex	Male	106	39	0.6951*	0.8963	0.5185
	Female	94	31			−1.5494
Comorbidities	Present	127	21	<0.0001	4.0594	2.2577
	Absent	73	49			−7.2989
Infections	Present	48	61	<0.0001	0.0466	0.0215
	Absent	152	09			−0.1008

* Statistically not significant.

Table 8 Univariate analysis of fluoroquinolone sensitive and resistant isolates of *K. oxytoca*.

Variables		Fq-S	Fq-R	*p*-value	Odd ratio	Range
Strains	HA	71	35	0.9656*	1.0143	0.5328
	CA	46	23			−1.9308
Sex	Male	70	37	0.6127*	0.8453	0.4410
	Female	47	21			−1.6203
Comorbidities	Present	65	19	0.0050	2.5658	1.3281
	Absent	52	39			−4.9570
Other infections	Present	27	41	<0.0001	0.1244	0.0611
	Absent	90	17			−0.2531

Fq-S, fluoroquinolone sensitive; Fq-R, fluoroquinolone resistant.
* Statistically not significant.

for *K. pneumoniae* (Tables 8 and 9). Males had 0.8453 (*K. oxytoca*) and 0.6320 (*K. pneumoniae*) times more risk of infection by fluoroquinolone-resistant isolates of both bacteria; however, these values were not statistically significant. People with non-infectious comorbidities were 2.5658 (*K. oxytoca*) and 1.7335 (*K. pneumoniae*) times more likely to become infected, and people with infectious comorbidities were 0.1244 (*K. oxytoca*) and 0.5455 (*K. pneumoniae*) times at more risk of infection with fluoroquinolone-resistant isolates. The data for the non-infectious or infectious comorbidities were highly significant for *K. oxytoca* and moderately significant for *K. pneumoniae* ($p < 0.05$). In conclusion, *K. pneumoniae* had a higher survival rate than *K. oxytoca*.

Discussion

In a surveillance period of 42 months, 445 strains of both *K. oxytoca* and *K. pneumoniae* were

Table 9 Univariate analysis of fluoroquinolone sensitive and resistant isolates of *K. pneumoniae*.

Variables		Fq-S	Fq-R	*p*-value	Odd ratio	Range
Strains	HA	120	41	0.3033*	1.3268	0.7744
	CA	75	34			−2.2733
Sex	Male	106	49	0.1037*	0.6320	0.3636
	Female	89	26			−1.0984
Comorbidities	Present	115	34	0.0446	1.7335	1.0134
	Absent	80	41			−2.9650
Related infections	Present	117	55	0.0428	0.5455	0.3034
	Absent	78	20			−0.9806

Fq-S, fluoroquinolone sensitive; Fq-R, fluoroquinolone resistant.
* Statistically not significant.

isolated from HA and CA clinical samples to determine the prevalence of β-lactam and fluoroquinolone-resistant strains. The antibiotic-resistant patterns of both *Klebsiella* species with 23 antibiotics from 9 different groups were ascertained, including 3GCs and a monobactam. Both strains were highly resistant to the β-lactam, cephalosporin and fluoroquinolone antibiotics. The β-lactam and fluoroquinolone-resistant strains of both species were equally prevalent. A univariate analysis revealed that patients with other bacterial infections had relatively higher chances of infection with ESBL-producing and fluoroquinolone-resistant strains of both *Klebsiella* species.

β-Lactamase enzymes have been classified by Ambler et al. [21] and Bush [22]. Ambler proposed four classes of enzymes, the C, A, B and D β-lactamases. Ambler class C belongs to group 1 (in a later classification) with 51 enzymes. Ambler classes 2a, 2b, 2be, 2br, 2c, 2d, 2e and 2f include 256 enzymes, such as broad-spectrum β-lactamase, carbenicillin hydrolyzing enzymes, clavulanate hydrolyzing oxacillin, carbapenem and cephalosporins. Ambler class B contains Bush group 3, consisting of metallo-β-lactamases, which are not inhibited by clavulanate. These are the primary groups of β-lactam antibiotics, with class D containing Bush group 4, which includes enzymes that are not grouped with the other enzyme classes. This group of enzymes was originally detected in the GN bacteria *P. aeruginosa*, *Citrobacter* sp. and *K. pneumoniae* and was later identified in GP bacteria. GNs with ESBL-producing capability remain susceptible to cefepime, a fourth-generation cephalosporin, and carbapenems.

The fluoroquinolone group is the preferred class of antibiotics for many ailments, such as respiratory, gastrointestinal, urogenital, intra-abdominal and skin infections caused by GN bacteria. The emergence of quinolone resistance was first reported in *K. pneumoniae* and has rapidly occurred in *S. aureus* and *P. aeruginosa* because it is plasmid borne [23,24]. By the beginning of this century, the majority of GN and GP bacteria acquired quinolone resistance [25,26]. Three elements are involved in quinolone resistance: *Qnr*, aminoglycoside acetyltransferase *AAC(6_)-Ib-cr*, and *OqxAB*, *QepA* [27]. However, nalidixic acid resistance is caused by the *Qnr*-protein, which reduces the susceptibility to low doses of fluoroquinolones [27]. In an animal study, lung infection with *K. pneumoniae* was treated with marbofloxacin (single shot administration or fractionated regimen over 4 days), a third-generation fluoroquinolone that successfully treated the infection, but resistant strains of the same bacterium emerged in the gut flora [28].

Carbapenem resistance occurs in both GP and GN bacteria. Carbapenem-resistant Enterobacteriaceae, particularly *Klebsiella* species, may be new superbugs because carbapenem is the last line of defense for drug-resistant *Klebsiella*. Failure of all other classes of antibiotics can be addressed by carbapenems only. Uncontrollable sepsis and bloodstream infections arising exclusively from drug-resistant Enterobacteriaceae are controlled exclusively by carbapenem antibiotics. The high meropenem resistance, 30—50% in the hospital evaluated in this study, is troubling. Despite the repeated warnings from clinical failures, our antimicrobial stewardship is inadequate. However, there are several controllable and uncontrollable factors involved in the emergence and spread of carbapenem resistance in the hospital and adjoining community. Hospitals everywhere, particularly in developing tropical countries, should focus on carbapenem resistance. An excellent study describes the primary genetic structures involved in the acquisition of the carbapenemase-resistant gene for all Enterobacteriaceae members and the

distribution of the genes responsible for resistance in the Ambler classes A, B and D [29]. The carbapenemase genes SME-1 to 3, NMC-A, IMI-2, GES-4,5,6, KPC-2 to 12, IMP-1 to -13, VIM-1 to -33, NDM-1 to -6, KHM-1, OXA 48, and OXA-181 have been described [29] and may be chromosomal or plasmid borne. The majority of carbapenemases are inhibited by clavulanate, tazobactam, sulbactam and NXL-104 but rarely by boronic acid, EDTA and NaCl.

In a Korean study, drug resistance in GN bacteria was observed for fluoroquinolone resistance in *E. coli* (27%), *P. aeruginosa* (33%) and *Acinetobacter* sp. (48%), whereas amikacin resistance occurred in *P. aeruginosa* (19%) and *Acinetobacter* sp. (37%). 3GC resistance was observed in *K. pneumoniae* (29%) [30]. Vancomycin-resistant *Enterococcus faecium* was also reported, and imipenem resistance in *Acinetobacter* sp. gradually increased in the hospital. *E. coli*, *Acinetobacter* sp., *P. aeruginosa* and *S. aureus* were the most prevalent MDR bacteria [30]. The detection of plasmid-mediated quinolone resistance in clinical isolates of hospitalized patients in Spain was reported for the *qnr*B5 and *qnr*B2 genes, both in *K. oxytoca* and *Enterobacter cloacae*, and the former gene showed higher prevalence [31]. A report from Iraq of a hospital based study of 259 under-5 children showed that 2.4% of the children had nosocomial diarrhea caused by *Enterobacter* sp., *P. aeruginosa* and *Citrobacter* sp. These incidences of diarrhea were linked to unclean food, contaminated bedsheets and personal hygiene affecting breast-feeding, and in all cases, contaminated hands played a significant role [32]. To decrease ESBL prevalence in a hospital or community, 3GC antibiotic use in non-hospitalized patients must also be reduced because the use of 2GC or 3GC increases the risk of fecal carriage of ESBL producers by 2—4-fold [33]. Gastrointestinal carriage of *K. pneumoniae* had been reported as the predisposing factor of pyogenic liver abscess in Taiwan. Molecular typing of isolated *K. pneumoniae* strains of the different serotypes K1 and K2 with *ramp*A genes concluded that virulence determinants result in this ailment [34]. Furthermore, *K. pneumoniae* serotypes K1 and K2 account for 98% of isolates from all countries and correspond to the serotypes isolated from liver abscesses in Asian countries [35]. Except for *Clostridium difficile*, nosocomial diarrhea is caused by other infectious agents, such as, norovirus and toxicogenic strains of *Clostridium perfringens*, *K. oxytoca*, *S. aureus* and *Bacteroides fragilis* [36]. Moreover, the fatality caused by *Klebsiella* sp. and other Enterobacteriaceae remains consistently high, with values of 16—37% in Thailand a decade ago. Recently the mortality of *Klebsiella* sp. and other Enterobacteriaceae members was determined to be 37.5% [37]. Recently, in Saint-Etienne, France, it was reported that ESBL-producing *K. pneumoniae* strains contained *blaCTXm-15* genes, causing bacterial resistance to ceftazidime, ciprofloxacin and tobramycin in ICU patients [38]. Antibiotic-associated hemorrhagic colitis was associated with *K. oxytoca* strains isolated from stool samples in Austria. Because *K. oxytoca* is an opportunistic pathogen, acquired drug resistance after antibiotic use may replace normal colonic micro flora with virulent strains. Therefore, adverse effects on the normal gut flora can occur after antibiotic therapy. However, *C. difficile* is an aggressive enteropathogen, and its MDR strains did not cause antibiotic-associated hemorrhagic colitis [39,40]. These infectious situations caused by *Klebsiella* sp. in diverse geographic zones demonstrate that these are clonally different and peripatetic.

In particular, elderly patients admitted to hospitals are generally multi-morbid and have reduced immune-competence. They are increasingly susceptible to antibiotic-resistant bacteria. ESBL-carrying patients have longer hospital stays and use of hospital devices for prolonged periods, which arise from and lead to illness from an infection with concomitant exposure to nosocomial infections with other pathogens. Eventually, newer antibiotics are be used [41,42], ultimately leading to multidrug-resistant bacteria.

In conclusion, among the Enterobacteriaceae, both species of *Klebsiella* had an alarming infection dynamics and were resistant to the 3GCs and fluoroquinolones. The *K. pneumoniae* strains were resistant to a greater number of antibiotics, and the percent resistance values for each antibiotic were higher with the isolated strains.

Funding

S. Rath is a Senior Research Fellow for a project from the Council of Scientific and Industrial Research, New Delhi [grant no. 21 (0859)/11/EMR-II], awarded to R.N. Padhy.

Competing interests

None declared.

Ethical approval

Not required.

Authors' contributions

S.R. and R.N.P. conceived and designed the study. S.R. collected, analyzed and interpreted the data. Both S.R. and R.N.P. were involved in drafting the manuscript. All authors read the manuscript and approved the final copy for submission.

Acknowledgements

We are grateful to the Department of Microbiology, IMS & Sum Hospital, Bhubaneswar, for use of the facilities. This work is a part of Ph.D. thesis in microbiology of S. Rath, Utkal University.

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